



## **ELISA PRODUCT INFORMATION & MANUAL**

### **Human Troponin I Type 3 (cardiac) ELISA Kit (Colorimetric)**

**NBP2-62177**

Enzyme-linked Immunosorbent Assay for  
quantitative detection of Human Troponin I.

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

# Product Manual

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# Product Manual

## TABLE OF CONTENTS



Please read entire booklet before proceeding with the assay.



Please contact Novus Biologicals Technical Support if necessary.



Carefully note the handling and storage conditions of each kit component.

<b>Introduction</b>	2
<b>Principles of Method</b>	2
<b>Intended Use</b>	3
<b>Storage and Stability</b>	3
<b>Chemical Hazard</b>	3
<b>Kit Contents</b>	3
<b>Other Materials Needed</b>	4
<b>Reagent Preparation</b>	5
Human Troponin I standard	5
Secondary Antibody	5
AV-HRP	6
Washing buffer	6
<b>Sample preparation</b>	7
<b>Assay Procedure</b>	7
<b>Characteristics</b>	9
Typical result	9
Sensitivity	10
Specificity	10
Precision	10
Recovery	10
<b>Troubleshooting</b>	11
<b>References</b>	13

# Product Manual

## INTRODUCTION

Troponin complex consists of three components: troponin I, T and C. The complex, along with tropomyosin, is located on the actin filament and is essential for the calcium-mediated regulation of skeletal and cardiac muscle contraction. Three isoforms of troponin I have been described for striated muscle. Two isoforms are characteristic for fast and slow skeletal fibers and one isoform for cardiac muscle.

Troponin I consists of 181-211 amino acid residues, and the cardiac isoform is larger due to the presence of an additional N-terminal peptide which has a biologically important function in the interactions of troponin I and troponin C. The main function of troponin I is the inhibition of actomyosin ATPase activity.

Cardiac troponin I and T are sensitive biomarkers of myocardial injury and have become central to the diagnosis of myocardial infarction. They are elevated in many clinical syndromes associated with direct myocardial injury, myocardial ischemia, or ventricular strain. They are also released in a number of clinical situations in which thrombotic complications of coronary artery disease. These situations include conditions like pulmonary embolism, sepsis, myocarditis, and acute stroke.

## PRINCIPLES OF METHOD

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human Troponin I. Samples are pipetted into these wells. Nonbound Troponin I and other components of the sample should be removed by washing, then biotin-conjugated monoclonal antibody specific to Troponin I added. In order to quantitatively determine the amount of Troponin I present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) should be added to each microplate well. The final step, a TMB-substrate solution added to each well. Finally, a sulfuric acid solution is added and the resulting yellow colored product is measured at 450nm. Since the increases in absorbency is directly proportional to the amount of captured Troponin I.

# Product Manual

## INTENDED USE

The Novus Biologicals Troponin I (human) ELISA kit is to be used for the *in vitro* quantitative determination of human troponin I in human serum, human plasma, buffered solution, or cell lysate.

**This kit has been configured for research use only and is not to be used in diagnostic procedures.**

## STORAGE AND STABILITY

All kit components of this kit are stable at 2 to 8°C. Any unused reconstituted standard should be discarded or frozen at -70°C.

**Standard can be frozen and thawed one time only without loss of immunoreactivity.**

## CHEMICAL HAZARD

**Stop solution:** This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.

**Standard protein and 2nd Antibody** containing Sodium Azide as a preservative.

## KIT CONTENTS

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Washing Buffer	2	(20X) 25 mL
Standard Protein	1	Glass vial (lyophilized)
Standard/Sample Dilution Buffer	1	25 mL
Secondary Antibody	1	Glass vial (lyophilized)
AV-HRP	1	(100X) 150 µL
Secondary Antibody/AV-HRP Dilution Buffer	1	25 mL
Substrate (TMB)	1	15 mL
Stop Solution	1	15 mL
Protocol booklet	1	-
Plate sealers	2	-

# Product Manual

1. **96 Well Plate:**  
Human Troponin I microtiter plate, one plate of 96 wells  
  
A plate using break-apart strips coated with a mouse monoclonal antibody specific to human Troponin I.
2. **Standard Protein:**  
Native human Troponin I.
3. **Secondary Antibody:**  
Biotinylated anti human Troponin I.
4. **AV-HRP**  
Avidin linked Horseradish Peroxidase (HRP, enzyme)
5. **Substrate (Stabilized chromogen)**  
Tetramethylbenzidine (TMB) solution
6. **Stop Solution**  
1N solution of sulfuric acid ( $H_2SO_4$ ).
7. **Plate sealer**  
Adhesive sheet.

*Do not mix or interchange different reagents from various kit lots.*

## OTHER MATERIALS NEEDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water
4. Data analysis and graphing software
5. Vortex mixer
6. Polypropylene tubes for diluting and aliquoting standard
7. Absorbent paper towels
8. Calibrated beakers and graduated cylinders of various sizes

# Product Manual

## REAGENT PREPARATION

### Human Troponin I standard

1. Reconstitute the lyophilized Human Troponin I standard by adding 1 mL of **Standard/Sample Dilution Buffer** to make the 2  $\mu$ g/mL standard stock solution. Allow solution to sit at RT for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Standard can be frozen and thawed one time only without loss of immunoreactivity.
2. Prepare 1 mL of 25 ng/mL top standard by adding 12.5  $\mu$ L of the above stock solution in 975  $\mu$ L of **Standard/Sample Dilution Buffer**. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (0.39 ng/mL ~ 25 ng/mL) as below. **Standard/Sample Dilution Buffer** serves as the zero standard (0 ng/ml).

Standard	Add	Into
25 ng/mL	12.5 $\mu$ L of the std. (2 $\mu$ g/mL)	987.5 $\mu$ L of the Standard/Sample Dilution Buffer
12.5 ng/mL	500 $\mu$ L of the std. (25 ng/mL)	500.0 $\mu$ L of the Standard/Sample Dilution Buffer
6.25 ng/mL	500 $\mu$ L of the std. (12.5 ng/mL)	500.0 $\mu$ L of the Standard/Sample Dilution Buffer
3.13 ng/mL	500 $\mu$ L of the std. (6.25 ng/mL)	500.0 $\mu$ L of the Standard/Sample Dilution Buffer
1.56 ng/mL	500 $\mu$ L of the std. (3.13 ng/mL)	500.0 $\mu$ L of the Standard/Sample Dilution Buffer
0.78 ng/mL	500 $\mu$ L of the std. (1.56 ng/mL)	500.0 $\mu$ L of the Standard/Sample Dilution Buffer
0.39 ng/mL	500 $\mu$ L of the std. (0.78 ng/mL)	500.0 $\mu$ L of the Standard/Sample Dilution Buffer
0 ng/mL	1.0 mL of the Standard/Sample Dilution Buffer	

### Secondary Antibody

100X secondary antibody solution can be made by adding 150  $\mu$ L secondary antibody/AV-HRP dilution buffer in the vial.

1. Equilibrate to room temperature, mix gently.
2. Mix 20  $\mu$ L **Secondary Antibody concentrated solution** (100X) + 1.98 mL **Secondary Antibody/AV-HRP dilution buffer**. (Sufficient for one strip, prepare more if necessary)  
Label as “*Working Secondary antibody Solution*”.
3. Return the unused **Secondary Antibody concentrated solution** to the refrigerator.

# Product Manual

## AV-HRP

1. Equilibrate to room temperature, mix gently.
2. Mix 20  $\mu$ L **AV-HRP concentrated solution (100X)** + 1.98 mL **Secondary Antibody/AV-HRP dilution buffer**. (Sufficient for one strip, prepare more if needed)  
Label as “Working AV-HRP Solution”.
3. Return the unused **AV-HRP concentrated solution** to the refrigerator.

## Washing buffer

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 0.5 volume **Wash buffer concentrate solution (20X)** + 9.5 volumes of deionized water. Label as “*Working Washing Solution*”.
3. Store both the concentrated and the Working Washing Solution in the refrigerator.

### \*Directions for washing

1. Fill the wells with 300  $\mu$ L of “Working Washing Buffer”.

Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

2. Incomplete washing will adversary affects the assay and renders false results.
3. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

# Product Manual

## SAMPLE PREPARATION

Blood should be collected by veinpuncture. For plasma samples, blood may be drawn into tubes containing sodium citrate or heparin, EDTA. The serum or plasma should be separated from the coagulated or packed cells by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze/thawing. Serum and plasma usually require at least *20 fold dilution* in the Standard/Sample Dilution Buffer. Please note that optimization may be required – each set of samples may be different.

## Assay Procedure

- Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
- All standards, controls and samples should be run in duplicate for confirmation of reproducibility.
- A standard curve must be run with each assay.
- If particulate matter is present in the analyte, centrifuge or filter prior to analysis.
- Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.

1. Determine the number of strips needed for assay. Insert these in the frame(s) for current use (Re-bag extra strips and frame. Refrigerate for further use).
2. For the standard curve, add 100 µL of the standard to the appropriate microtiter wells. Add 100 µL of the **Standard/Sample Dilution Buffer** to zero wells.
3. Serum and plasma require at least *20 fold dilution* in the **Standard/Sample Dilution Buffer**. And add 100 µL of samples to each well.
4. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
5. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See “*Directions for washing*”).
6. Pipette 100 µL of “Working Secondary Antibody Solution” into each well.

# Product Manual

7. Cover the plate with the plate cover and incubate for 1 hour at room temperature.
8. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
9. Add 100  $\mu$ L "Working AV-HRP Solution" to each well.
10. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
11. Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
12. Add 100  $\mu$ L of **Substrate** to each well. The liquid in the wells should begin to turn blue.
13. Incubate the plate at room temperature.
  - Do not cover the plate with aluminum foil, or color may develop.

*The incubation time for chromogen substrate is often determined by the microtiter plate reader used. O.D. values should be monitored and the substrate reaction stopped before O.D. of the positive wells exceeds the limits of the instrument. O.D. values at 450 nm can only be read after the Stop Solution has been added to each well.*
  - Because the Substrate is light sensitive, avoid prolonged exposure to light for the remaining Substrate solution.
  - Typically, reaction is stopped 5~10 minutes after treatment of Substrate, but this time can be adjusted as the user desires.
14. Add 100  $\mu$ L of Stop Solution to each well. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm. Read the plate within 20 minutes of adding the Stop Solution.
16. Plot the absorbance of the standard against the standard concentration (Optimally, the background absorbance can be subtracted from all data points, including standards, unknowns and controls, prior to plotting.). Draw a smooth curve through these points to construct the standard curve.

# Product Manual

17. Read the human Troponin I concentrations for the unknown samples and controls from the standard curve plotted in step 16. Multiply value(s) obtained for the unknown sample by the dilution factor (Samples producing signals greater than that of the highest standard should be further diluted in the **Standard/Sample Dilution Buffer**).

## CHARACTERISTICS

### Typical results

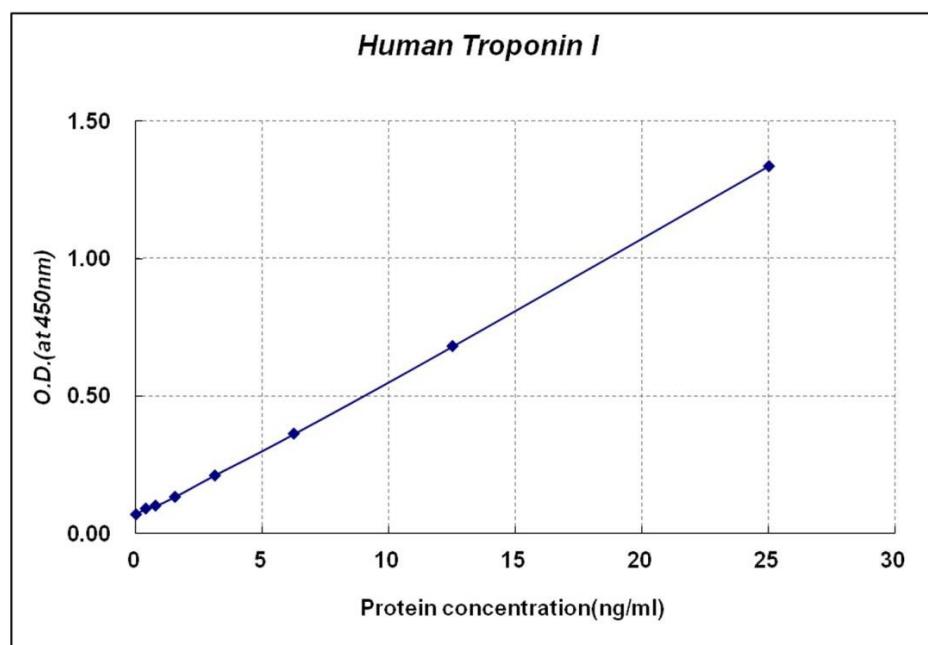
The standard curve below is for illustration only and should not be used to calculate results in your assay.

*A standard curve must be run with each assay.*

Standard Human Troponin I (ng/mL)	Optical Density (at 450 nm)
0	0.070
0.39	0.090
0.78	0.100
1.56	0.134
3.13	0.212
6.25	0.362
12.50	0.680
25.00	1.337

### Limitations

- Do not extrapolate the standard curve beyond the 25 ng/mL standard point.
- Other buffers and matrices have not been investigated.
- The rate of degradation of native human Troponin I in various matrices has not been investigated.



# Product Manual

## Sensitivity

The minimal detectable dose of human Troponin I was calculated to be 0.38 ng/mL, by subtracting two standard deviations from the mean of 10 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

## Specificity

The following substances have been tested and found to have no cross-reactivity: cardiac Troponin T and cardiac Troponin C. Muscle Troponin I had slight cross-reactivity.

## Precision

### 1. Within-Run (Intra-Assay)

(n=6)

Mean (ng/mL)	SD (ng/mL)	CV (%)
3.11	0.19	6.0
6.02	0.35	5.9
12.28	1.09	8.9
23.15	0.85	3.7

### 2. Between-Run (Inter-Assay)

(n=3)

Mean (ng/mL)	SD (ng/mL)	CV (%)
3.01	0.01	0.4
5.83	0.08	1.4
11.32	0.37	3.3
25.6	1.57	6.1

## Recovery

Recovery on addition is 98.3~104.2 % (mean 100.8%)

Recovery on dilution is 99.3~103.9 % (mean 100.7 %)

For the recovery assay, a known amount of the ELISA standard is spiked into serially-diluted normal human serum and run in the assay. Any endogenous levels are taken into account and then % recovery of the exogenously spike standard is determined.

# Product Manual

## TROUBLESHOOTING

Problem	Possible Cause	Solution
High signal and background in all wells	Insufficient washing	Increase number of washes Increase time of soaking between in wash
	Too much AV-HRP	Check dilution, titration
	Incubation time too long	Reduce incubation time
	Development time too long	Decrease the incubation time before the stop solution is added
No signal	Reagent added in incorrect order, or incorrectly prepared	Review protocol
	Standard has gone bad (If there is a signal in the sample wells)	Check the condition of stored standard
	Assay was conducted from an incorrect starting point	Allow reagents to come to 20~30°C before performing assay
Too much signal – whole plate turned uniformly blue	Insufficient washing – unbound AV-HRP remaining	Increase number of washes carefully
	Too much AV-HRP	Check dilution
	Plate sealer or reservoir reused, resulting in presence of residual Streptavidin -HRP	Use fresh plate sealer and reagent reservoir for each step

# Product Manual

Problem	Possible Cause	Solution
Standard curve achieved but poor discrimination between point	Plate not developed long enough	Increase substrate solution incubation time
	Improper calculation of standard curve dilution	Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	Sample matrix is masking detection	More diluted sample recommended
Samples are reading too high, but standard curve is fine	Samples contain protein levels above assay range	Dilute samples and run again
Edge effect	Uneven temperature around work surface	Avoid incubating plate in areas where environmental conditions vary Use plate sealer

# Product Manual

## REFERENCES

1. Fromm RE Jr et al. Cardiac troponins in the intensive care unit: common causes of increased levels and interpretation. 2007, Crit Care Med. 35(2):584-588.
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# Product Manual

## NOTES